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RESEARCH PAPER

Sodium alginate and carboxymethyl cellulose-based composite coating enriched *Lactiplantibacillus plantarum* reduced anthracnose disease and maintained the quality of "Cat Hoa Loc" mango fruits

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Abstract

Anthracnose, caused by *Colletotrichum gloeosporioides* species complex and identified through internal transcribed spacer (ITS) region sequence homology, poses a major challenge to postharvest "Cat Hoa Loc" mangoes by reducing fruit quality, shelf life, and export potential. This study evaluated the efficacy of a 2.0% sodium alginate (SA) and 0.25% carboxymethyl cellulose (CMC) coating, enriched with *Lactiplantibacillus plantarum* (10^6 CFU mL^{-1}), in controlling anthracnose and preserving fruit quality. *In vitro*, *Lactiplantibacillus plantarum* isolate LDC11 inhibited the *C. gloeosporioides* species complex mycelial and spore growth by 42.5% and 53.8%, respectively. The combined treatment of the SA-CMC coating with *Lactiplantibacillus plantarum* isolate LDC11 reduced disease incidence by 3-fold and disease severity by 2.89-fold, extending the storage period by 3 days at 25 °C, compared to the control. This treatment significantly preserved fruit quality, maintaining high levels of firmness, vitamin C, phenolic compounds, and chlorophyll, while also slowing respiration rates, delaying the decline in titratable acidity (TA), and impeding increases in total soluble solids (TSS). Weight loss was unaffected. Overall, the SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11 provides a promising, eco-friendly postharvest approach that could be applied in industrial mango preservation and export processing.

Keywords

Anthracnose disease, biocontrol, edible coating, *Lactiplantibacillus plantarum*, *Mangifera indica* L., postharvest management, sodium alginate - carboxymethyl cellulose

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Introduction

Mango (*Mangifera indica* L.) cv. Cat Hoa Loc is a popular fruit in Vietnam, valued for its exceptional taste, appearance, and high nutritional content, including phytochemicals, vitamin C, carotenoids, polyphenols, and dietary fiber (Thinh and Kunasakdakul 2013). However, "Cat Hoa Loc" mangoes are highly perishable and particularly susceptible to anthracnose disease caused by *Colletotrichum* sp. during storage, transportation, and marketing (Thinh and Kunasakdakul 2013; Danh et al. 2021). Physiological changes in postharvest mangoes, such as chemical alterations, chlorophyll degradation, carotenoid metabolism, cell wall breakdown, and enzymatic hydrolysis of starch into sugars, adversely affect their shelf life (Nguyen et al. 2021a, 2021b). Therefore, effective postharvest treatments are essential to mitigate anthracnose and maintain quality.

Edible coatings based on polysaccharides are widely used to protect agricultural products from physical, chemical, physiological, and biological deterioration, thereby extending the shelf life of perishable items during storage (Panahirad et al. 2021). Previous studies have shown that coatings made from SA or CMC can reduce physiological changes and preserve quality in various fruits, including apples (Olivas et al. 2007), plums (Valero et al. 2013; Panahirad et al. 2020), kiwifruit (Liu et al. 2020), mandarins (Baswal et al. 2020), rose apples (Duong et al. 2022), guavas (Kumar et al. 2021), mangoes (Ali et al. 2022a), and bananas (Ali et al. 2022b). Truc et al. (2023) reported that a 2.0% SA-0.25% CMC composite coating significantly reduced physiological changes and preserved the quality of "Cat Hoa Loc" mangoes stored at 25 °C. However, the antifungal efficacy of this coating against anthracnose remains limited, highlighting the need for enhancements.

In recent years, antagonistic agents such as bacteria and yeasts have been effectively employed to control postharvest diseases in fruits (Arroyave-Toro et al. 2017; Chen et al. 2020; Shi et al. 2021; Thi et al. 2023). For instance, Thi et al. (2023) demonstrated that lactic acid bacteria (LAB), specifically *Lactiplantibacillus plantarum* isolate LDC11, could reduce postharvest anthracnose in papaya without affecting the fruit's physiological characteristics. Thus, the combination of antagonistic agents with an edible coating can enhance their adherence to the fruit surface. A previous study showed that candelilla wax coating combined with *Bacillus subtilis* HFC103 reduced postharvest disease caused by *Rhizopus stolonifer* in strawberries (Oregel-Zamudio et al. 2017). Similarly, Fan et al. (2009) found that combining SA coatings with *Cryptococcus laurentii* yeast effectively reduced disease severity and maintained the quality of harvested strawberries. Moreover, a study demonstrated that CMC and SA based coatings combined with brewer's yeast improved the sensory attributes and overall quality of grapes (Yinzhe and Shaoying 2013). Additionally, Álvarez et al. (2021) confirmed that exopolysaccharide-based edible coatings combined with *Lactiplantibacillus plantarum* isolate LDC11 exhibited antifungal activity while maintaining postharvest quality in cherry tomatoes.

Despite these promising findings, limited information is available regarding the effects of sodium alginate-carboxymethyl cellulose (SA-CMC) coatings enriched with *Lactiplantibacillus plantarum* isolate LDC11 on anthracnose in mangoes. Therefore, this study aims to report the impact of SA-CMC coatings enriched with *Lactiplantibacillus plantarum* isolate LDC11 on anthracnose disease in "Cat Hoa Loc" mangoes, evaluated through both *in vitro* and *in vivo* assays, as well as its effects on fruit quality maintenance.

Materials and methods

Isolation and identification of *Colletotrichum* sp.

Colletotrichum species were isolated from naturally infected "Cat Hoa Loc" mango fruits exhibiting typical anthracnose symptoms, characterized by large, sunken, circular lesions with dark brown to black discoloration. Fungal isolation was performed using the tissue transplant method described by Danh et al. (2021). Infected mangoes were first rinsed with distilled water, and tissues from the margin between healthy and diseased areas were excised into 1 × 1 cm pieces. The tissue segments were surface sterilized in 70% ethanol for 90 seconds, rinsed three times with sterile distilled water, and blotted dry on sterile tissue paper. The sterilized tissues were aseptically transferred onto potato dextrose agar (PDA) plates (200 g L^{-1} potato, 20 g L^{-1} glucose, and 20 g L^{-1} agar; pH: 7.0). The plates were incubated at 30 °C for 3–5 days. Identification of the *Colletotrichum* species was based on morphological characteristics, polymerase chain reaction (PCR) amplification, and sequencing of the ITS region using primers ITS1: 5'-TCC GTA GGT GAA CCT GCG G-3' and ITS4: 5'-TCC TCC GCT TAT TGA TAT GC-3' (White et al. 1990). PCR products were purified and sequenced by Macrogen Inc. (Korea; www.macrogen.com). The pathogenicity of *Colletotrichum* sp. isolate was confirmed on healthy mango fruits using Koch's postulates.

Lactiplantibacillus plantarum LDC11 bacteria isolation

The *Lactiplantibacillus plantarum* isolate LDC11, previously obtained from fermented vegetables, was identified through morphological observation and molecular characterization using the PCR technique (Thi et al. 2023).

Antagonistic activity of *L. plantarum* isolates LDC 11 against *Colletotrichum* sp.

The mycelial and spore antagonistic assays were conducted to evaluate the antifungal activity of *Lactiplantibacillus plantarum* isolate LDC11 against *Colletotrichum* sp. Mycelial growth inhibition was assessed using the dual culture method with minor modifications. *Lactiplantibacillus plantarum*

isolate LDC11 was inoculated in two parallel 2-cm lines on potato dextrose agar (PDA) plates, and a 5-mm mycelial plug, obtained using a cork borer, was placed at the center between the bacterial lines. Plates containing only mycelial plugs served as negative controls. All plates were incubated at 30 °C for 5–7 days, and inhibitory activity was evaluated by measuring the reduction in mycelial growth compared to the control. The percentage of mycelial growth inhibition was calculated according to the method described by Mpeluza et al. (2023).

The dual culture overlay method was utilized to evaluate the spore-inhibitory activity of *Lactiplantibacillus plantarum* isolate LDC11 against *Colletotrichum* sp. (Magnusson et al. 2003). Briefly, *Lactiplantibacillus plantarum* isolate LDC11 was inoculated in two parallel 2-cm lines on De Man-Rogosa-Sharpe (MRS) agar plates (Himedia, India) and anaerobically incubated at 30 °C for 24 hours. The plates were then overlaid with 2 mL of PDA medium (0.7% agar) supplemented with *Colletotrichum* spores at a density of 10^6 spores mL^{-1}. Afterward, the plates were incubated at 30 °C for 48 hours. The diameter (d) of the inhibition zone was recorded and compared to the control. Spore antagonistic activity (%) was calculated following the method outlined by Muhialdin et al. (2018).

Preparation of SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11

The effect of the SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11 on anthracnose incidence and the postharvest quality of mango fruits was investigated using methods adapted from Shahrampour et al. (2020) and Oregel-Zamudio et al. (2017). Green-mature mangoes (80–90 days after fruit set) were harvested from a Viet GAP farm in the Dong Thap province, Vietnam, and transported to the laboratory within 1 hour under ambient conditions (30 ± 2 °C). The selected fruits were uniform in color and weight (330–375 g per fruit) and free from defects. Prior to treatment, the mangoes were surface disinfected with a 0.1 g L^{-1} sodium hypochlorite solution for 5 minutes and then rinsed with distilled water. Previous studies have reported that a 2.0% SA-0.25% CMC coating effectively reduced physiological changes and maintained the quality of mangoes during storage at 25 °C (Truc et al. 2023).

The 2.0% SA-0.25% CMC film-forming solution was prepared according to the procedure suggested by Truc et al. (2023). Briefly, 2.0% (w/v) sodium alginate (General Purpose Grade, Fisher Chemical, UK) was dissolved in distilled water and stirred for 1 hour at 60 °C. Subsequently, 0.25% (w/v) sodium carboxymethyl cellulose (Xilong Scientific, China) was added and mixed with a magnetic stirring bar until fully dissolved. Thereafter, 2% glycerol, 0.2% Tween-80, and 1.0% calcium chloride (w/v) were incorporated, and the solution was stirred for an additional hour to obtain a homogeneous mixture. Air bubbles were removed by centrifugation at 3,000 × g for 3 minutes, and the resulting SA-CMC solution was used for subsequent experiments.

Lactiplantibacillus plantarum isolate LDC11 was cultured in an MRS medium and incubated at 30 °C for 24 hours. The bacterial density was determined by spectrophotometric measurement at 600 nm, where an optical density ($OD_{600\ nm}$) of 1.0 corresponds to a bacterial density of 10^8 CFU mL^{-1} (Kim et al. 2021). Finally, the *Lactiplantibacillus plantarum* isolate LDC11 suspension was adjusted to a concentration of 10^6 CFU mL^{-1} using either the 2.0% SA-0.25% CMC solution or MRS medium.

Mango treatment with SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11

Mango fruit samples were divided into four groups, each containing 55 fruits. Each group was dipped for 1 minute in one of the following solutions: (1) control treatment, consisting of MRS broth without coating and live cell of *Lactiplantibacillus plantarum* isolate LDC11 (Control); (2) 2.0% SA-0.25% CMC coating enriched with live cell of *Lactiplantibacillus plantarum* isolate LDC11 at a concentration of 10^6 CFU mL^{-1} (SA-CMC-*L. plantarum*); (3) 2.0% SA-0.25% CMC coating alone (SA-CMC); and (4) MRS broth enriched with live cell of *Lactiplantibacillus plantarum* isolate LDC11 at a concentration of 10^6 CFU mL^{-1} (*L. plantarum*). After treatment, all fruit samples were stored at 25 °C (RH = 60 ± 5%) in polyethylene bags (22 × 18 cm) with 32 holes (Ø 0.6 mm) for 12 days. Fruits were randomly selected for quality evaluation on the initial day and then at 3-day intervals throughout the storage period.

Evaluation of disease incidence and severity

The incidence of anthracnose disease was determined as the percentage of infected fruits within each treatment group. Disease severity was assessed by evaluating the total decayed area on each fruit, following a modified five-point scale based on Hofman et al. (1997). The scale was defined as follows: 0 = no disease symptoms, 1 = < 5% of the fruit surface affected, 2 = 5.1–15% affected, 3 = 15.1–30% affected, 4 = 30.1–50% affected and 5 = > 50% affected.

Determination of physiological changes

Weight loss

Weight loss of mango fruit was determined as a percentage loss of the initial total weight, as described by Almenar et al. (2006).

Color change

The color changes in the peel of mangoes were measured using a colorimeter (Hunter Lab, MH-C800 4500L, USA) every 3 days. Three points of fruit were

selected for color measurement. Ten fruits (replicates) were used for each treatment, and the values of a* were recorded (Truc et al. 2021).

Respiration rate

The respiration rate was measured using a carbon dioxide sensor (CO_2-BTA, Vernier, USA) connected to a personal computer, following the method described by Raudienė et al. (2017). The fruits were incubated in a tightly sealed plastic container (3.0 L volume) for 30 minutes at 25 °C. Carbon dioxide concentration and temperature within the sample box were recorded and reported as mg CO_2 kg^{-1} h^{-1}. Each treatment was replicated three times.

Total chlorophyll content

Chlorophyll was extracted using N,N-dimethylformamide at 4 °C in the dark for 24 hours. The solution was then filtered through Whatman No. 1 filter paper. The absorption values for chlorophyll content were measured at 664 nm and 647 nm using a spectrophotometer (BioSpectrometer Basic D30, Germany) and expressed as mg kg^{-1} of fresh weight (Moran 1982).

Determination of fruit quality

Fruit firmness

The firmness of the mango flesh was measured using a texture analyzer (Food Technology TMS-Pro, USA) equipped with a 5-mm diameter probe. Measurements were taken in the middle section of the fruit after removing 1 mm of the peel, with the probe penetrating to a depth of 10 mm at a rate of 10 mm per minute. Firmness values were expressed in Newtons (N) (Truc et al. 2021).

Total soluble solids and Titratable acidity

Fresh juice was extracted from the middle part of the flesh using a mortar and pestle and then filtered through a clean cloth. Total soluble solids (TSS) were measured with a handheld refractometer (Atago, Japan) and reported as a percentage. Titratable acidity (TA) was determined by titrating 5 mL of mango juice with 0.1 N NaOH, using phenolphthalein as the indicator. TA content was expressed as a percentage of citric acid.

Vitamin C content

Vitamin C content was measured according to the method described by Nguyen et al. (2021b). Briefly, 5 g of mango flesh was homogenized with 20 mL of cold 5% metaphosphoric acid and filtered through Whatman No. 1 filter paper at 4 °C. The reaction mixture was prepared by mixing 0.4 mL of the filtered solution with 0.2 mL of 0.02% di-indophenol. Next, 0.4 mL of 2% thiourea and 0.2 mL of 2% dinitrophenylhydrazine were added, followed by incubation at 50 °C in a water bath (WNB-7, Germany) for 1 hour. After incubation, 1 mL of 85% sulfuric acid was added, and the mixture was allowed to stand at an ambient temperature for 30 minutes. The absorbance of the vitamin C solution was measured at 540 nm using a spectrophotometer (BioSpectrometer Basic D30, Germany). The results were calibrated using freshly prepared L-ascorbic acid standard solutions (0–100 μg mL^{-1}). A calibration curve ($R^2 > 0.99$) was established, and the data were expressed as grams of ascorbic acid per kilogram of fresh weight. All measurements were conducted in triplicate for biological replicates and in duplicate for each technical replicate.

Total phenolic content

The crude extract was prepared following the method described by Ribeiro et al. (2008) with some modifications. Mango peel powder was mixed with 60% methanol (v/v), homogenized, and then centrifuged at 15,000 × g for 20 minutes at 4 °C. The supernatant was used to determine the total phenolic content using the Folin - Ciocalteu method, as outlined by Singleton et al. (1998). For the reaction mixture, 0.05 mL of the extract was combined with 0.25 mL of Folin–Ciocalteu reagent, followed by adding 0.75 mL of 7.5% sodium carbonate solution and 2 mL of distilled water. The mixture was vortexed and incubated in a water bath at 40 °C for 30 minutes. The absorbance was measured at 750 nm using a spectrophotometer (BioSpectrometer Basic D30, Germany). Quantification was performed using freshly prepared gallic acid standard solutions (0–1000 μg mL^{-1}) for calibration. The calibration curve ($R^2 > 0.99$) was constructed, and results were expressed as grams of gallic acid equivalents (GAE) per kilogram of fresh weight. Each experiment was conducted using three biological replicates, and each measurement was repeated twice for technical replication.

Statistical analysis

The experimental data were analyzed using statistical analysis software (SAS), version 9.0 (SAS Institute, Cary, NC, USA), following a completely randomized design. One-way analysis of variance (ANOVA) and LSD multiple range tests were employed to compare treatment differences at a 95% confidence level. Data are presented as mean ± standard deviation. The BLASTn tool was used to assess the similarity of fungal strains in the NCBI database. A neighbor-joining phylogenetic tree was constructed using MEGA 5.1 software and the Kimura-2 model, with 1,000 replications.

Results

Isolation and identification of *Colletotrichum* sp.

A pathogenic fungal isolate, designated CHL1, was obtained from mango fruits exhibiting typical anthracnose symptoms (Fig. 1A). The colony of this isolate grown on PDA medium developed dense, ivory-white aerial mycelia that flocculated at the center and gradually thinned

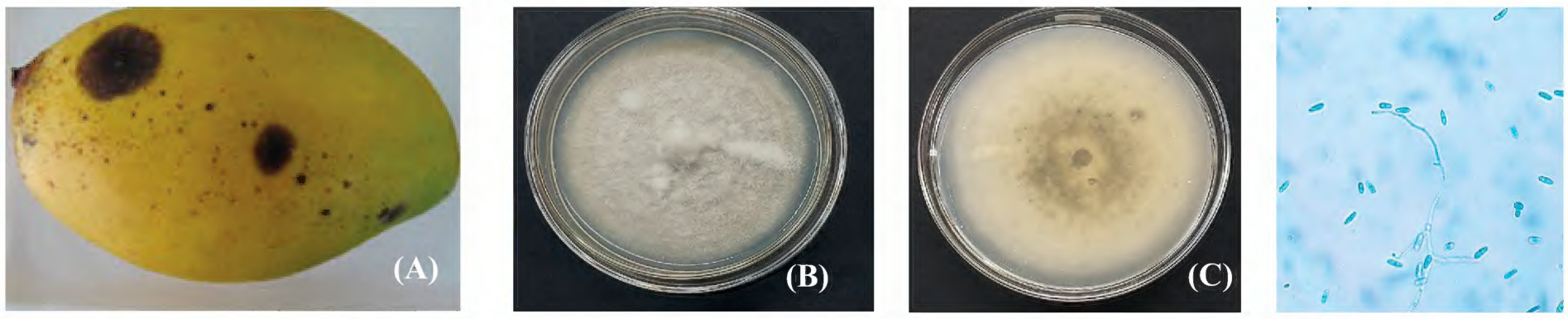

Figure 1. Isolation of the fungus *C. gloeosporioides* species complex isolate CHL1. **A.** naturally infected mango fruit; **B, C.** fungal colony on PDA medium (top view and view below the petri dish) after incubation for 7 d; **D.** spore under the microscope (100X).

toward the edges. The reverse side of the colony displayed a light olive color in the center, with pale white margins (Fig. 1B, C). Microscopic observation revealed that the fungal spores were cylindrical, with one end pointed and the other rounded, while the hyphae were predominantly simple and lacked septa (Fig. 1D). The pathogenicity of CHL1 was confirmed using Koch's postulates. The results indicated that CHL1 caused infection in mango fruits, with typical anthracnose symptoms appearing within 3–5 days after inoculation (data not shown). Sequencing results demonstrated that CHL1 had 97.50% homology with *Colletotrichum gloeosporioides* species complex isolate FJAT-32130 (OR726223.1) in the NCBI database. The phylogenetic tree illustrated that the CHL1 isolate clustered separately (Fig. 2). Thus, the CHL1 isolate was identified as belonging to *C. gloeosporioides* species complex, which is capable of infecting mango fruits and causing anthracnose symptoms.

Antagonistic activity of *Lactiplantibacillus plantarum* against *C. gloeosporioides* species complex isolate CHL1

The antifungal activity of *Lactiplantibacillus plantarum* isolate LDC11 against *C. gloeosporioides* species complex isolate CHL1 is presented in Fig. 3. The results indicated that *Lactiplantibacillus plantarum* isolate LDC11 exhibited the highest mycelial antagonistic activity against CHL1 on Day 4, achieving a 42.5% inhibition of fungal growth, which then decreased significantly to 18.3% by Day 7 (Fig. 4A). As shown in Fig. 4B, the spore antagonistic activity of *Lactiplantibacillus plantarum* isolate LDC11 resulted in 53.8% and 44.6% inhibition compared to the control on Days 2 and 3, respectively. These results indicate that *Lactiplantibacillus plantarum* isolate LDC11 could effectively control anthracnose in mango fruits.

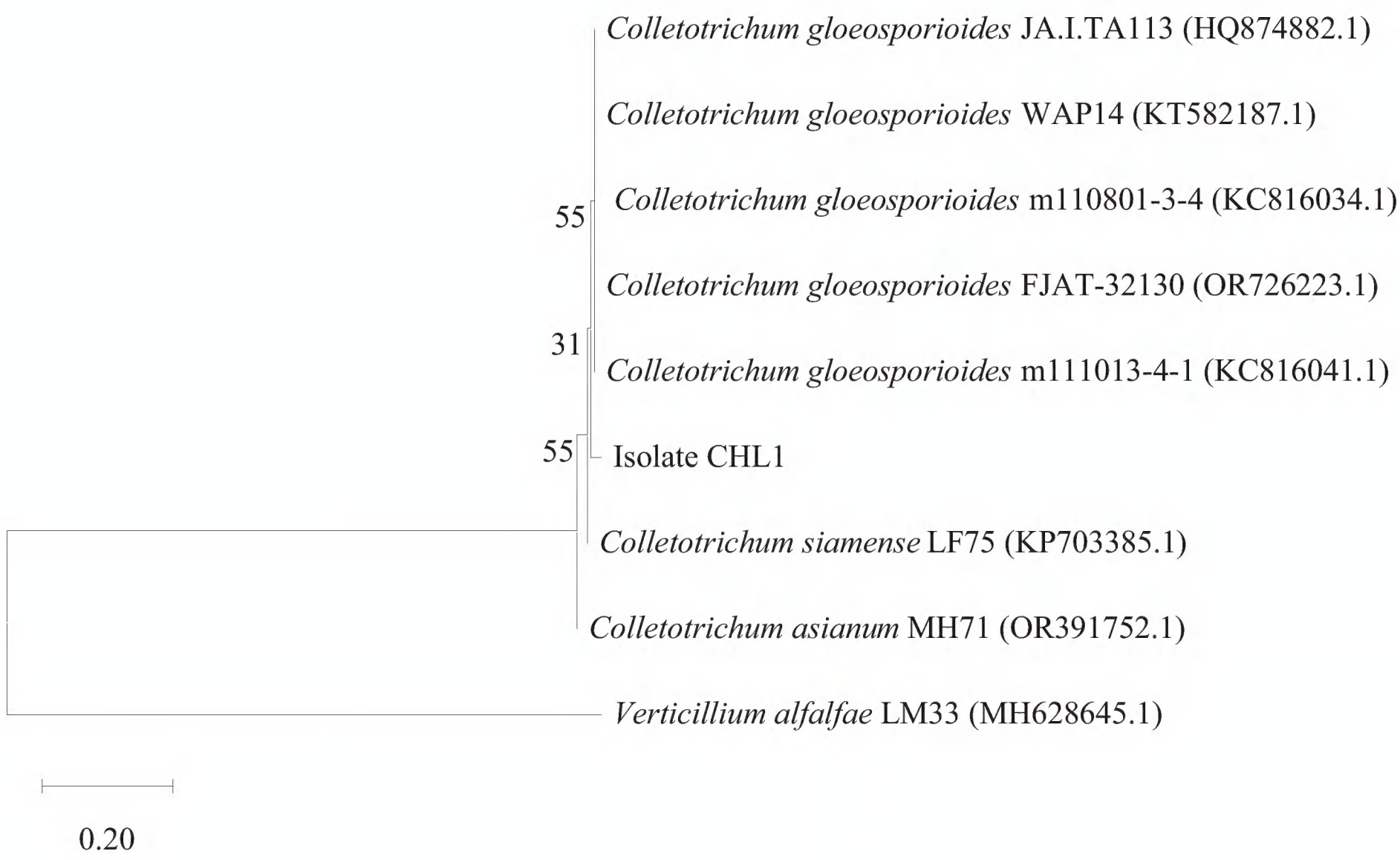

Figure 2. Phylogenetic tree of *Colletotrichum gloeosporioides* species complex Isolate CHL1 based on ITS regions of ribosomal DNA sequences (bootstrap values are given at branching points).

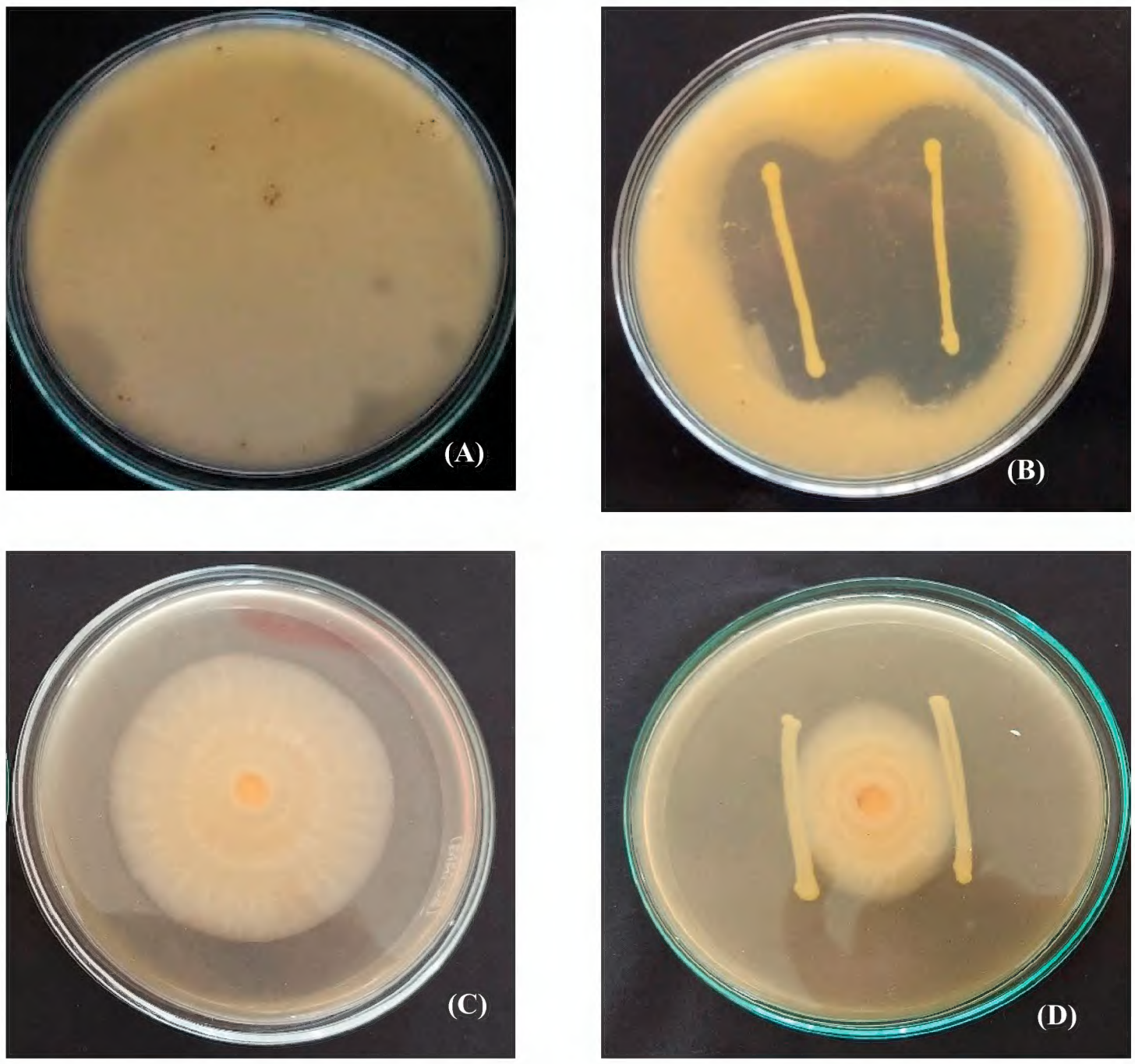

Figure 3. Antagonistic activity of *Lactiplantibacillus plantarum* isolate LDC11 against spore and mycelial growth of *C. gloeosporioides* species complex isolate CHL1. **A.** spore of the *C. gloeosporioides* species complex isolate CHL1 (control); **B.** Antagonistic activity of *Lactiplantibacillus plantarum* isolate LDC11 against the spores of the *C. gloeosporioides* isolate CHL1 on Day 2; **C.** The mycelium of the *C. gloeosporioides* species complex isolate CHL1 (control), and **D.** Antagonistic activity of *Lactiplantibacillus plantarum* isolate LDC11 against the mycelium of the *C. gloeosporioides* species complex isolate CHL1 on Day 4.

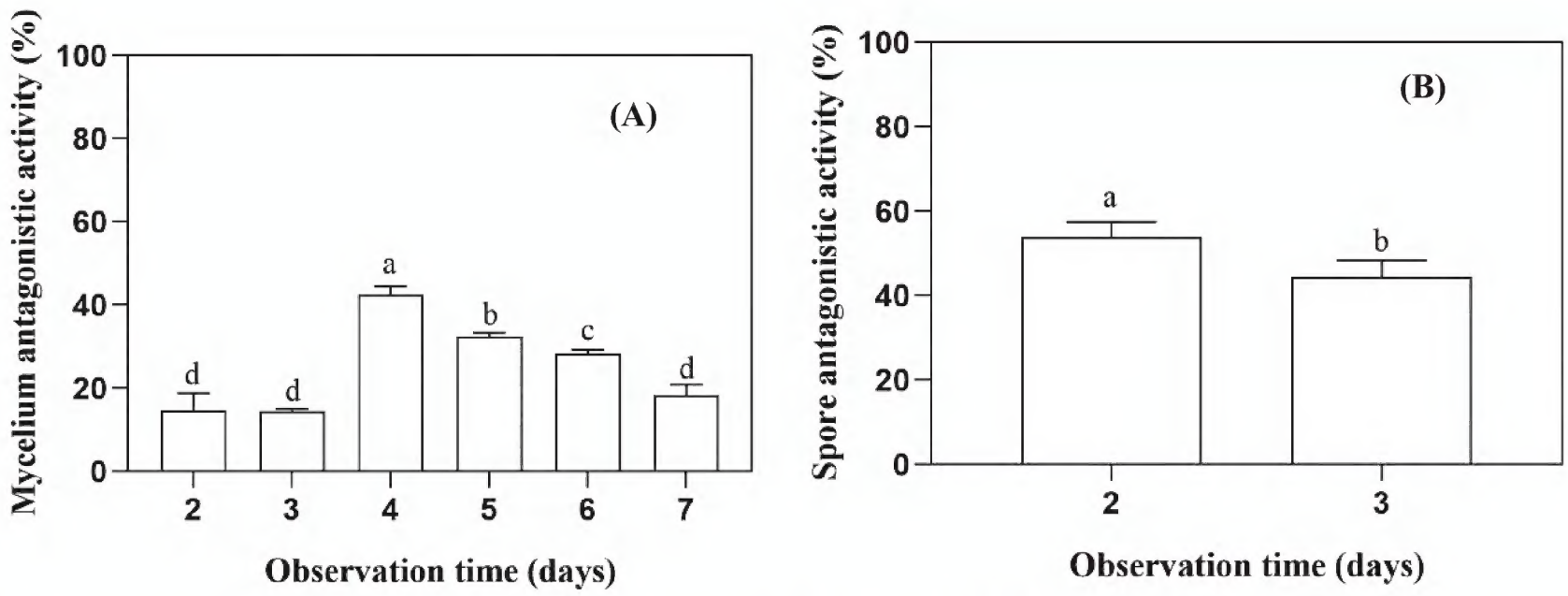

Figure 4. Antagonistic activity of *Lactiplantibacillus plantarum* isolate LDC11 against mycelial (**A**) and spore (**B**) of the *C. gloeosporioides* species complex isolate CHL1. Data are expressed as mean ± standard deviation. Different letters within each storage day indicate significant differences based on the LSD multiple range test ($P < 0.05$).

Effect of SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11 on disease incidence and severity

The effect of the SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11 on the incidence and severity of anthracnose disease in naturally infected mango fruits while stored at 25 °C is shown in Fig. 5. The combined treatment of SA-CMC coating and *Lactiplantibacillus plantarum* isolate LDC11 significantly reduced both disease incidence and severity compared to the control. Disease progression was markedly faster in the control fruits than in the

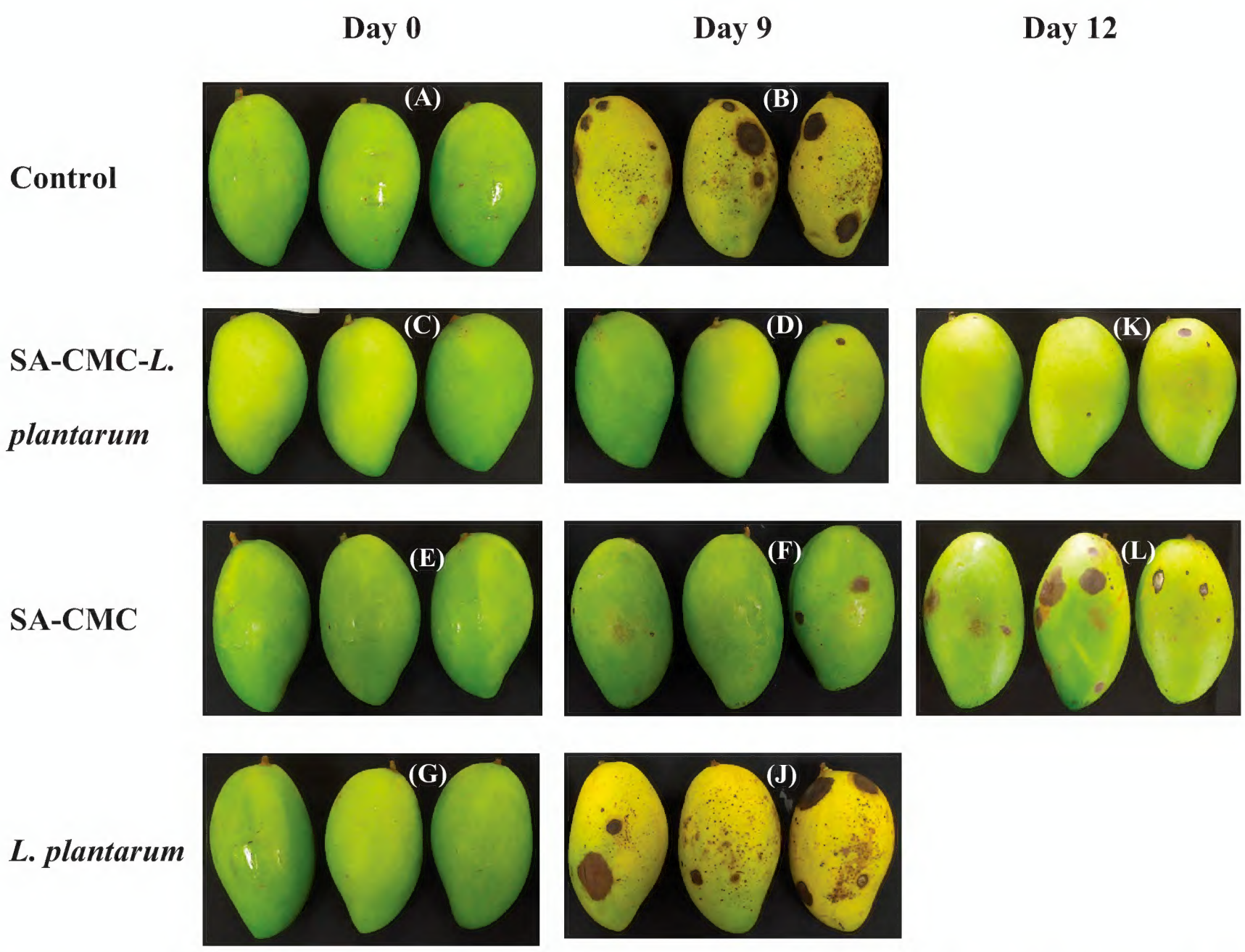

Figure 5. Effect of SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11 on the visual appearance of mangoes. Fruit samples were then stored at 25 °C for 12 d.

treated groups. On Day 3, fruits treated with the SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11 and *Lactiplantibacillus plantarum* isolate LDC11 alone showed no infection symptoms, whereas disease incidences of 40.0% and 33.3% were recorded for the SA-CMC coating and control treatments, respectively. After 6 days of storage, disease incidence reached 100% in control, followed by *Lactiplantibacillus plantarum* isolate LDC11 (93.33%), the SA-CMC coating (53.33%), and the SA-CMC coating combined with *Lactiplantibacillus plantarum* isolate LDC11 (33.33%). By the end of the storage period (12 days), all treatments exhibited 100% disease incidence (Fig. 6A). Similarly, the SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11 resulted in the lowest disease severity (1.73 scores), followed by the SA-CMC coating treatment (2.2 scores), the *Lactiplantibacillus plantarum* isolate LDC11 treatment alone (5.0 scores), and the control treatment (5.0 scores) on Day 12 (Fig. 6B). These findings indicate that the SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11 effectively suppresses the development of anthracnose disease in "Cat Hoa Loc" mango fruits.

Effect of SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11 on physiological changes

The respiration rate is a key indicator for evaluating the postharvest shelf life of fresh produce, as a higher respiration rate accelerates senescence. Statistical analysis revealed that the combined treatment of SA-CMC coating and *Lactiplantibacillus plantarum* isolate LDC11, as well as the SA-CMC coating alone, significantly reduced the respiration rate compared to the control and *Lactiplantibacillus plantarum* isolate LDC11 treatments from Days 3 to 9 of storage. However, no significant differences in respiration rates were observed among all treatments on the initial and final days of storage (Fig. 7A). Weight loss in mango fruits increased progressively during storage, with no significant differences between treatments (Fig. 7B).

Color change is a reliable parameter for assessing fruit ripening. The results indicated that the SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11 and the SA-CMC coating alone exhibited lower a* values compared to the control and *Lactiplantibacillus plantarum* isolate LDC11 treatments from Days 3 to 9 of storage. However, no significant differences in a* values of the peel were noted among all treatments on Day 0 and Day 12 (Fig. 7C).

Chlorophyll, the green pigment indicative of fresh and healthy fruit, showed a decreasing trend in all treatments throughout the storage period. On Days 0 and 12, there were no significant differences in total chlorophyll content among treatments. Nevertheless, statistical analysis demonstrated that the SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11 and the SA-CMC coating alone maintained

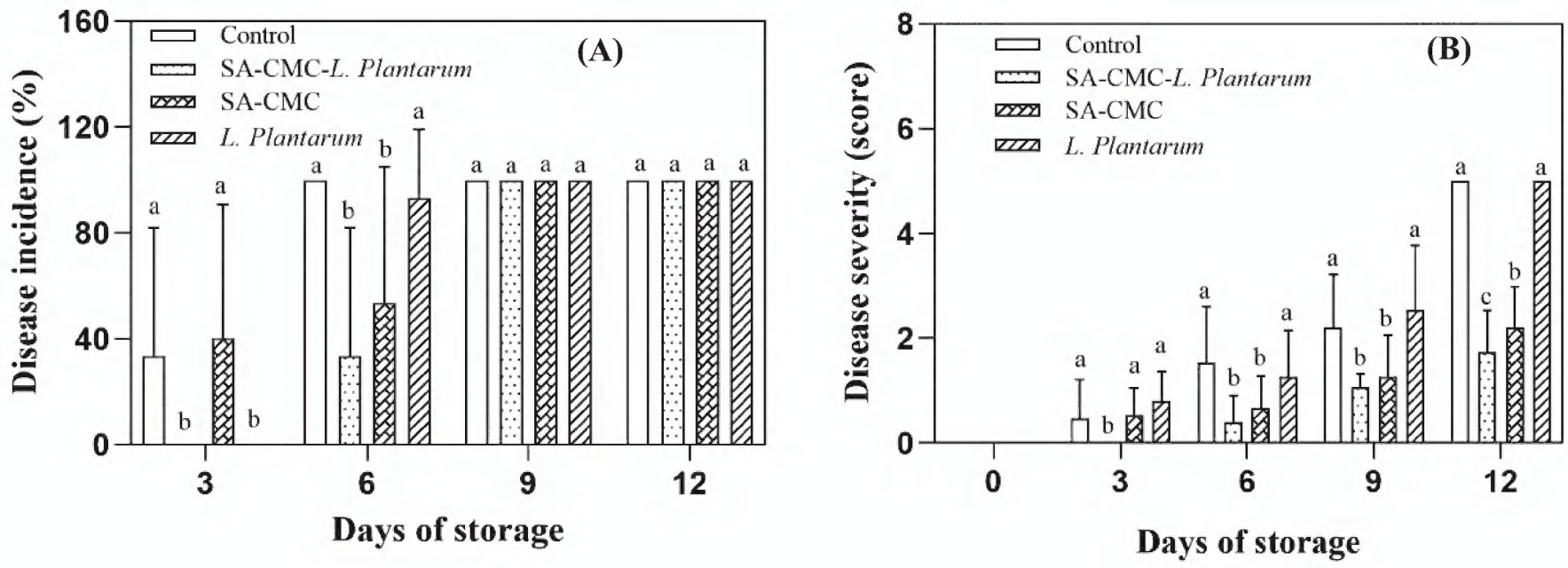

Figure 6. Effect of SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11 on disease incidence (**A**) and severity (**B**). Fruit samples were then stored at 25 °C for 12 d. Data are expressed as mean ± standard deviation (n = 15). Different letters within each storage day indicate significant differences based on the LSD multiple range test ($P < 0.05$).

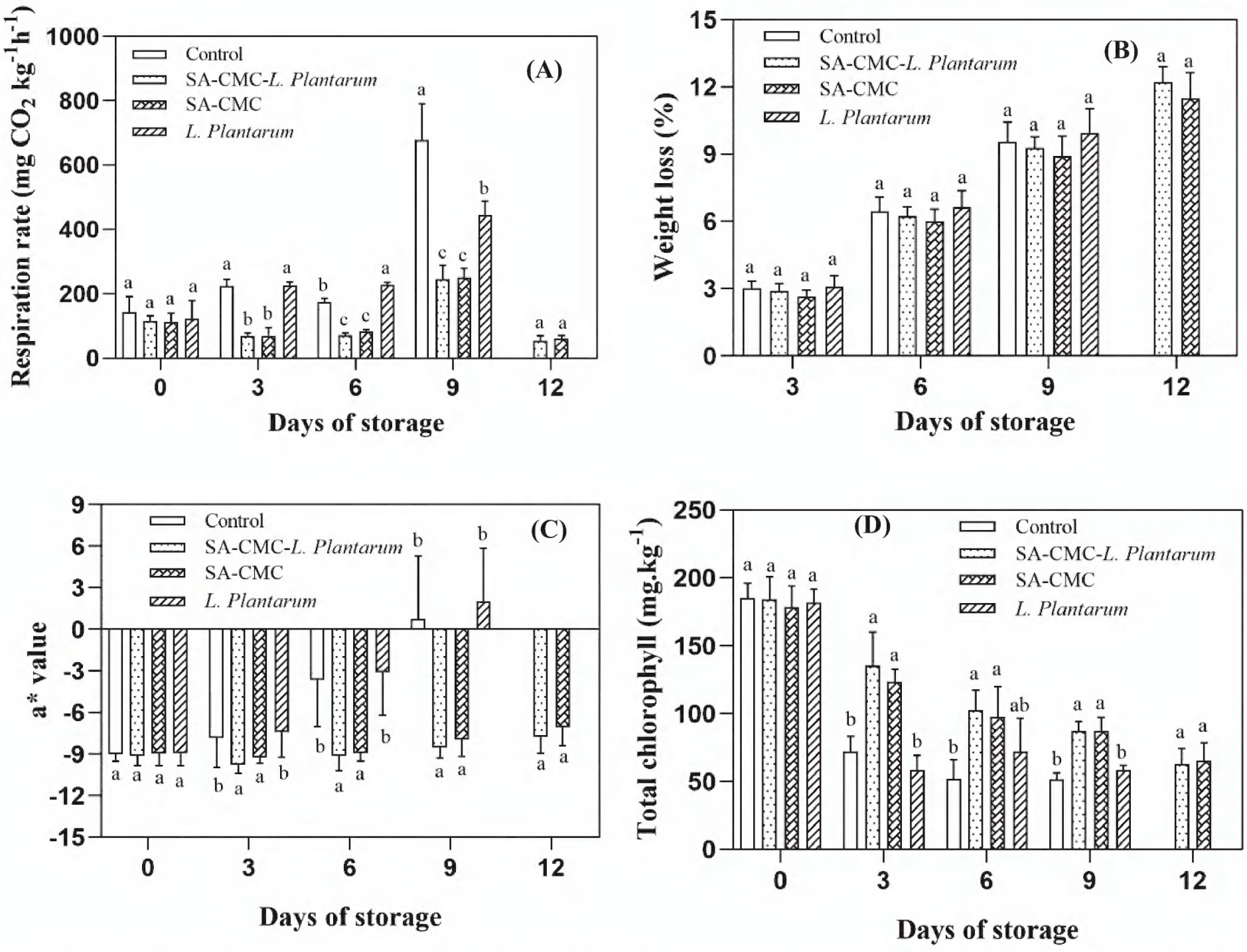

Figure 7. Effect of SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11 on respiration rate (**A**), weight loss (**B**), a* values (**C**), and total chlorophyll content (**D**). Fruit samples were stored at 25 °C for 12 d. Data are expressed as mean ± standard deviation (n = 3). Different letters within each storage day indicate significant differences based on the LSD multiple range test ($P < 0.05$).

significantly higher total chlorophyll content compared to the control and *Lactiplantibacillus plantarum* isolate LDC11 treatments from Days 3 to 9 of storage. This result correlated with the changes observed in peel color (a* values), suggesting that the SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11 and the SA-CMC coating alone effectively preserved the green color of the mango peel (Fig. 7D).

Effect of SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11 on fruit quality

The results showed that mango fruit firmness decreased sharply from Day 0 to Day 6 across all treatments, followed by a slight decline from Days 9 to 12 (Fig. 8A). The combined SA-CMC coating and *Lactiplantibacillus plantarum* isolate LDC11 treatment maintained the highest firmness values from Days 3 to 12, followed by the SA-CMC coating alone, *Lactiplantibacillus plantarum* isolate LDC11 treatment, and the control, respectively. By the end of the storage period, fruits in the control and *Lactiplantibacillus plantarum* isolate LDC11 treatment groups were completely decayed, whereas those treated with SA-CMC coating enriched with *Lactiplantibacillus plantarum*

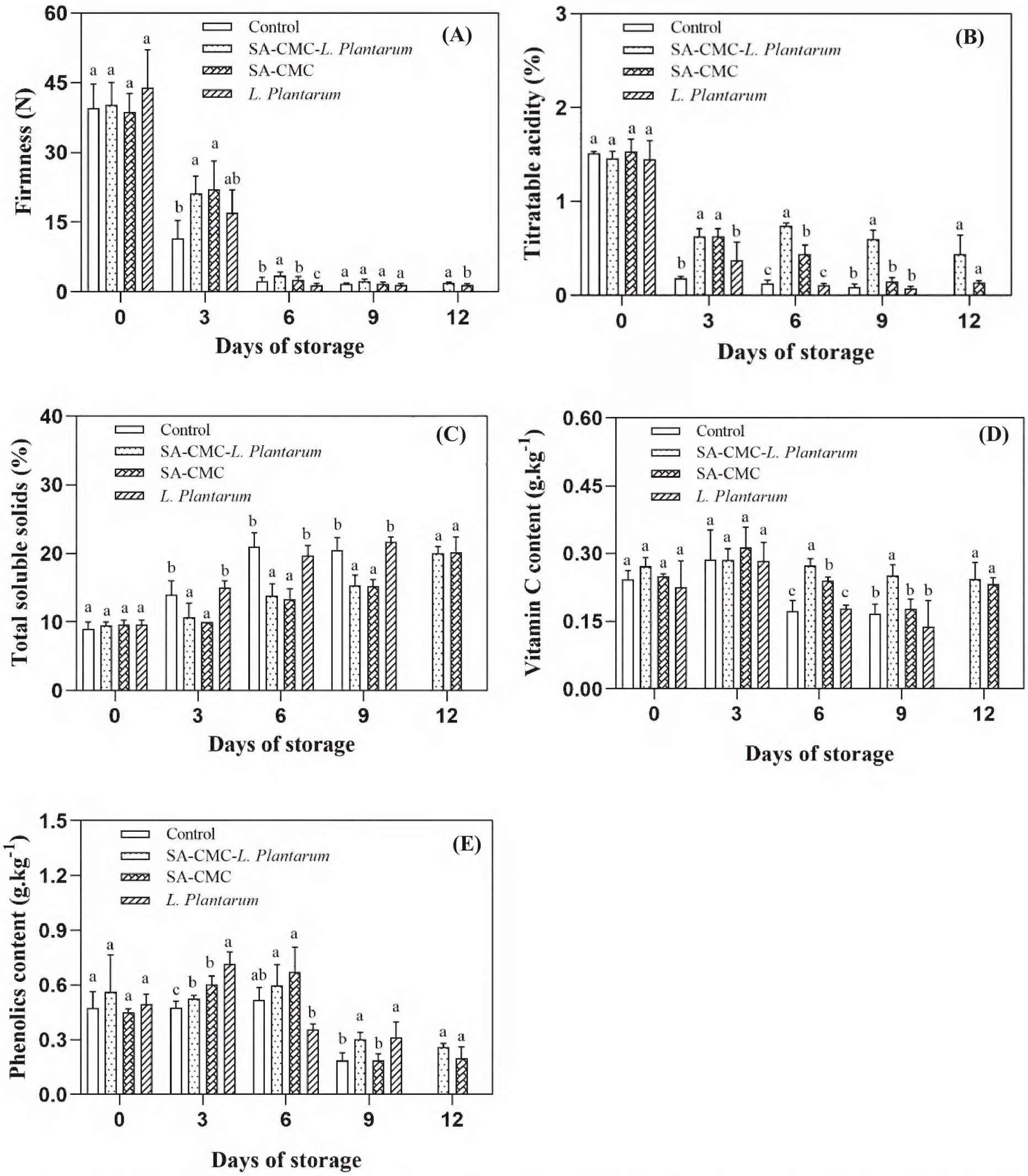

Figure 8. Effect of SA-CMC coating enriched with *L. plantarum* isolate LDC11 on fruit quality; firmness (**A**), titratable acidity (**B**), total soluble solids (**C**), vitamin C (**D**) and total phenolic content (**E**). Fruit samples were stored at 25 °C for 12 d. Data are expressed as mean ± standard deviation. Different letters within each storage day indicate significant differences based on the LSD multiple range test ($P < 0.05$).

isolate LDC11 and SA-CMC coating alone exhibited firmness values of 1.87 N and 1.49 N, respectively.

TA values in all treatments decreased during storage, with no significant differences among treatments on Day 0. From Days 3 to 12, fruits treated with SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11 exhibited the highest TA values, followed by the SA-CMC coating alone, control, and *Lactiplantibacillus plantarum* isolate LDC11 treatment (Fig. 8B).

TSS content increased slightly during storage for all treatments. Statistical analysis revealed that the SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11 and the SA-CMC coating alone significantly delayed the rise in TSS values compared to the control and *Lactiplantibacillus plantarum* isolate LDC11 treatments from Days 3 to 9. However, by the end of the storage period, there were no significant differences in the TSS content between the SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11 and the SA-CMC coating alone (Fig. 8C).

Vitamin C, an important antioxidant, showed no significant differences among treatments from Days 0 to 3. However, from Days 6 to 9, the SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11 had a significantly higher vitamin C content compared to the other treatments (Fig. 8D).

The phenolic content in mango fruit showed a slight increase on Day 0, peaking on Day 3 across all treatments before declining. No significant differences in phenolic content were observed between treatments on Days 0 and 12. Fruits treated with SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11 and those with only *Lactiplantibacillus plantarum* isolate LDC11 had significantly higher phenolic content compared to the control on Days 3 and 9. However, on Day 6, no significant differences were found between the SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11 and the SA-CMC coating alone compared to the control (Fig. 8E).

Discussion

Anthracnose disease, caused by *Colletotrichum* sp., is a major contributor to economic losses in postharvest mango production. In this study, the isolate CHL1 of *C. gloeosporioides* species complex isolate CHL1 was identified as the causal agent of anthracnose affecting "Cat Hoa Loc" mango fruits. To mitigate this disease, an SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11 was applied as a postharvest treatment, and its effects were evaluated through both *in vitro* and *in vivo* experiments. *In vitro* results showed that *Lactiplantibacillus plantarum* isolate LDC11 inhibited 42.5% of mycelial growth and 53.8% of spore germination of *C. gloeosporioides* compared to the control. Consistently, previous research reported that *Lactiplantibacillus plantarum* isolate LDC11 treatment reduced the growth of *C. fructicola* YCH09, another anthracnose pathogen in papaya, by 62.8% (Thi et al. 2023). Numerous studies have documented the ability of LAB, including *Lactobacillus paracasi*, *Pediococcus pentosaceus*, *Lactobacillus amylovorus*, *Lactobacillus fermentum*, and *Lactobacillus reuteri*, to inhibit the growth of various fungal pathogens. This antifungal activity is primarily attributed to the production of bioactive compounds such as lactic acid, phenolic acids, organic acids, reuterin, and antimicrobial peptides (AMPS LR14) (Trias et al. 2008; Ghosh 2015; Husain et al. 2017; Nasrollahzadeh et al. 2022). *Lactobacillus plantarum* has been reported to inhibit fungi isolated from cherry tomatoes through the production of lactic and acetic acids (Melo et al. 2021). Therefore, the effectiveness of *Lactiplantibacillus plantarum* isolate LDC11 against *C. gloeosporioides* species complex isolate CHL1 in this study likely due to the production of these antifungal compounds.

The results revealed that the SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11 treatment reduced disease incidence by threefold on Day 6 of storage, followed by a 1.87-fold reduction for the SA-CMC coating alone and a 1.07-fold reduction for *Lactiplantibacillus plantarum* isolate LDC11 alone. Similarly, the combined treatment also lowered disease severity by 2.89-fold at the end of storage, compared to the control, with the SA-CMC coating alone and *Lactiplantibacillus plantarum* isolate LDC11 alone showing reductions of 2.27-fold and 1.00-fold, respectively. The enhanced resistance to anthracnose in mango fruits following the SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11 treatment is attributed to the synergistic effects of both treatments.

Numerous studies have demonstrated that polysaccharides-based edible coatings, such as carboxymethyl cellulose, chitosan, aloe vera, and gum arabic, can reduce disease incidence and severity by inducing the activity of defense enzymes, including chitinase, β-1,3-glucanase, peroxidase, and phenylalanine ammonia-lyase in various fruits (Wang and Gao 2013; Bill et al. 2014; Chen et al. 2017). Thi et al. (2023) reported that *Lactiplantibacillus plantarum* isolate LDC11 treatment reduced disease incidence and severity in papaya fruits. Although, *Lactiplantibacillus plantarum* isolate LDC11 exhibited strong antifungal activity against *C. gloeosporioides* species complex isolate CHL1 *in vitro*, its application alone was ineffective in controlling anthracnose in mangoes *in vivo*, likely due to the limited nutrient availability on the mango surface compared to the nutrient-rich *in vitro* environment, as well as lower humidity conditions that hindered *Lactiplantibacillus plantarum* isolate LDC11's effectiveness. Nevertheless, the SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11 reduced anthracnose disease more effectively than either treatment alone or the control, supporting the synergy between the two treatments. These findings are consistent with recent reports demonstrating the synergistic benefits of probiotic-based edible coatings in managing postharvest fungal diseases. Menéndez-Cañamares et al. (2024) found that an alginate coating with *Bacillus subtilis* SB8 markedly reduced *Botrytis cinerea* infection in strawberries while maintaining the fruit's quality. Similarly, Huang et al. (2024) also showed that a gelatin film incorporating

Lactiplantibacillus plantarum SCSL-1 effectively inhibited *Colletotrichum gloeosporioides* on mangoes and kept the fruit fresh during storage. In this study, the mechanisms underlying the observed disease reduction were not directly investigated. It is conceivable that the combination of practical LAB cells and the polysaccharide-based coating contributed synergistically to disease suppression. The metabolic movement of live *Lactiplantibacillus plantarum* isolate LDC11 may have created natural acids and other antimicrobial metabolites, whereas the coating lattice likely given a physical obstruction that decreased oxygen dissemination and dampness misfortune. This double activity may clarify the lower infection frequency and slower natural product weakening watched within the LAB-enriched coating treatment. Encourage thinks about are required to confirm these speculations through practicality measures, pH estimations, and comparison with cell-free supernatant.

Mango fruits, as climacteric produce, rapidly lose quality during ripening. Therefore, the impact of the SA-CMC coating and *Lactiplantibacillus plantarum* isolate LDC11 treatment on the physiological and quality attributes of postharvest mangoes was also examined. The results indicated that both the SA-CMC coating alone and its combination with *Lactiplantibacillus plantarum* isolate LDC11 delayed the respiratory climacteric peak compared to the control, whereas the *Lactiplantibacillus plantarum* isolate LDC11 treatment alone had no significant effect on the respiration rate. This delay can be attributed to the SA-CMC coating forming a thin film that acts as a barrier against gas and water transpiration, thereby reducing oxygen availability for respiration and limiting carbon dioxide diffusion from the fruit tissue (Moalemiyan et al. 2012). Previous studies have shown that SA and CMC can effectively reduce respiration rates in postharvest peaches (Maftoonazad et al. 2008). Álvarez et al. (2021) reported that exopolysaccharides combined with LAB reduced the respiration rate of postharvest cherry tomatoes. However, the SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11 did not significantly affect weight loss during storage, which is consistent with findings that certain coatings, such as chitosan and carboxymethyl cellulose, do not significantly influence weight loss in citrus fruits (Arnon et al. 2014). This result may be due to the poor water barrier of SA-CMC coating (Panahirad et al. 2020; Salama and Abdel-Aziz 2021). Our result agrees with Ranjith et al. (2021) who reported that LAB treatment did not affect the weight loss of mango fruits.

During storage, the color of mango peels transitions from green to yellow as ripening progresses, which is associated with chlorophyll degradation (Medlicott et al. 1986). This study demonstrated that the SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11 delayed the green-to-yellow color change in mango peels, likely by helping to maintain chlorophyll content during storage. Similar observations were reported by Nguyen et al. (2021b), who found that retention of green color was closely associated with higher chlorophyll levels. However, the *Lactiplantibacillus plantarum* isolate LDC11 treatment alone did not affect color change compared to the control, a finding echoed in previous research on papaya (Thi et al. 2023).

Chlorophyll, a key indicator of fruit freshness and quality, can be preserved by edible coatings, which create a modified atmosphere that limits oxygen diffusion and reduces respiration and ethylene production, thereby delays chlorophyll degradation (Maftoonazad et al. 2008; Hong et al. 2012; Han et al. 2014). The decline in chlorophyll content during storage is generally associated with the activity of chlorophyll-degrading enzymes. Previous studies have demonstrated that chlorophyll levels decrease in parallel with increased activity of enzymes such as chlorophyllase, magnesium dechelatase, pheophytinase, and chlorophyll-degrading peroxidase (Chl-POX) during storage (Nguyen et al. 2021; Pongsri et al. 2021). The present study found that the mango fruits treated with SA-CMC coating alone and the combination with *Lactiplantibacillus plantarum* isolate LDC11 maintained higher total chlorophyll content than the control throughout storage, potentially due to reduced activity of chlorophyll-degrading enzymes associated with lower respiration rates. However, further investigation is needed to explore the relationship between SA-CMC treatment, enzyme activity, and chlorophyll degradation.

The SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11 treatment significantly preserved fruit firmness. High firmness is correlated with elevated levels of cellulose, hemicellulose, and water-insoluble pectin, along with low levels of water-soluble pectin and reduced activity of softening enzymes (Nguyen et al. 2021a). Previous studies have indicated that CMC coatings effectively maintain fruit firmness in guavas and plums (Panahirad et al. 2020; Kumar et al. 2021). Ma et al. (2021) noted that shellac coatings reduced firmness loss in mangoes by inhibiting polygalacturonase activity, while CMC coatings decreased softening enzyme activities in postharvest mangoes and bananas (Ali et al. 2022a, 2022b). Our findings align with those of Álvarez et al. (2021), who reported maintained firmness in tomatoes treated with exopolysaccharides and LAB.

In this study, both the SA-CMC coating alone and the combination with *Lactiplantibacillus plantarum* isolate LDC11 resulted in lower TSS content than the control, consistent with reports of CMC coatings suppressing TSS increases in mangoes and guavas (Ali et al. 2022a, 2022b). However, other studies indicated that *Lactiplantibacillus plantarum* isolate LDC11 treatment did not significantly influence TSS content in papaya and mango (Thi et al. 2023; Ranjith et al. 2021). The TA in the SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11 treatment was higher than in the control, possibly due to reduced respiration rates delaying mango ripening. This finding is supported by Álvarez et al. (2021), who found that exopolysaccharide-based coatings combined with LAB resulted in higher TA levels than controls in harvested cherry tomatoes.

Vitamin C, or ascorbic acid, plays a vital role in maintaining health, supporting the immune system, and promoting skin integrity. Research indicates that vitamin C reduction is linked to respiration rates, reactive oxygen

species (ROS), and ascorbic peroxidase activity (Fenech et al. 2019). High respiration rates, particularly under stress or elevated temperatures, can lead to quicker depletion of vitamin C (Mditshwa et al. 2017). Saleem et al. (2022) found a correlation between high respiration rates and low vitamin C content. In this study, mangoes treated with the SA-CMC coating, either alone or enriched with *Lactiplantibacillus plantarum* isolate LDC11, retained higher vitamin C levels compared to the control. This preservation effect is likely attributed to the gas barrier formed by the SA-CMC coating, which limits oxygen exposure and consequently reduces oxidative degradation of ascorbic acid. Reports suggest that coatings can also reduce ascorbate peroxidase activity, thereby helping to maintain vitamin C levels in strawberries and plums (Wang and Gao 2013; Panahirad et al. 2020). Further study found that an SA-CMC coating combined with yeasts resulted in higher vitamin C content compared to the control in grapes (Yinzhe and Shaoying 2013). However, this effect was not observed with the *Lactiplantibacillus plantarum* isolate LDC11 alone treatment, which aligns with the findings of Thi et al. (2023) that indicated no significant impact on vitamin C in papaya.

This research confirmed that both the SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11 and the SA-CMC coating alone significantly increased total phenolic content compared to the control. Martínez-Castellanos et al. (2011) reported high phenolic concentrations in LAB-treated litchi, while Mpeluza et al. (2023) demonstrated that *Lactobacillus plantarum*-inoculated avocado induced phenylalanine-ammonia lyase (PAL) gene expression and increased phenolic compounds. Further studies indicated that chitosan coating treatments maintained higher phenolic levels in fruit (Wang and Gao 2013). Moreover, edible coatings based on SA, CMC, chitosan, and aloe vera have been shown to increase phenolic compounds and PAL enzyme activity in various fruits, including mango (Rastegar et al. 2019), sweet cherry (Díaz-Mula et al. 2012), guava (Kumar et al. 2021), and avocado (Bill et al. 2014).

Conclusion

This study confirmed that the *C. gloeosporioides* species complex isolate CHL1 is the causal agent of anthracnose disease in "Cat Hoa Loc" mango fruits. The *Lactiplantibacillus plantarum* LDC11, isolated from traditional fermented vegetables, exhibited notable antifungal activity, inhibiting 42.5% of mycelial growth and 53.8% of spore germination of the *C. gloeosporioides* species complex. The application of an SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11 (10^6 CFU mL^{-1}) reduced disease incidence by threefold and disease severity by 2.89-fold in mango fruits. This treatment delayed fruit ripening, preserved firmness and titratable acidity, reduced respiration, color changes and total soluble solids, and maintained higher levels of vitamin C, phenolics, and chlorophyll, without significantly affecting weight loss. Overall, the SA-CMC coating enriched with *Lactiplantibacillus plantarum* isolate LDC11 effectively alleviate anthracnose, maintain quality, and extend the shelf life of postharvest "Cat Hoa Loc" mangoes. Moreover, this bio-based coating shows promising potential for industrial-scale mango preservation and sustainable postharvest management.

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